

U.S. Patent Application No. 10/070,882
Supplemental Amendment and Response to Non-Final Office Action
Page 2

Amendments to the Specification

On Page 1, above the first paragraph, please insert the following section heading:

CROSS REFERENCE TO RELATED APPLICATIONS

On Page 1, above the first paragraph and below the newly inserted section heading described above, please insert the following new paragraph:

This application is a 35 U.S.C. §371 of PCT/GB96/00571, filed March 13, 1996.

On Page 1, above the first paragraph and below the newly inserted section described above, please insert the following section heading:

BACKGROUND OF THE INVENTION

On Page 4, between lines 4 and 6, please insert the following section heading:

OBJECTS OF THE INVENTION

On Page 4, between lines 31 and 33, insert the following section heading:

DETAILED DESCRIPTION OF THE INVENTION

On Page 12, between lines 3 and 5, insert the following section heading:

BRIEF DESCRIPTION OF THE DRAWINGS

U.S. Patent Application No. 10/070,882
Supplemental Amendment and Response to Non-Final Office Action
Page 3

Please replace the paragraph on page 12, lines 16-18, as follows:

~~Figure 3 shows~~ Figures 3a and 3b show graphs illustrating IgG serum antibody levels in mice to the carrier bacterium, (Figure 3a) and to the F1 antigen (Figure 3b), 21, 28 and 98 days after immunization;

Please replace the paragraph on page 12, lines 33-35, as follows:

~~Figure 7 shows~~ Figures 7a and 7b show the results of elispot analysis of Peyer's patch cells and in particular the IgA response against F1 antigen (Figure 7a) and Salmonella (Figure 7b).

Please replace the paragraph on page 19, lines 1-26 as follows:

All measurements of antibody levels in individual animals were determined in duplicate. For enzyme-linked ~~immunosorbent~~ immunosorbent assays (ELISAs) to determine IgG and IgA titres, 96-well microtiter plates were coated overnight at 4°C either with 50µl 5µg/ml purified F1-antigen (Miller J, et al., FEMS Microbiology and Immunology 1998;21:213-21) in PBS or with 50µl 6µg/ml *S. typhimurium* SL3261 lysate in PBS, prepared as follows. Bacteria were grown statically overnight at 37°C, prior to harvesting and resuspension in PBS to an approximate concentration of 1×10^{10} cfu/ml. Cells were heat-killed in a boiling water bath for 30 ~~minutes~~ min, cooled on ice and then sonicated on ice for 6 pulses of 30 ~~seconds~~ s. Total protein concentration was determined by a BCA protein assay (Pierce and Warriner, Chester, UK). Plates were blocked for 1 ~~hour~~ h at 37°C with PBS containing 1% (w/v) skimmed milk powder (BLOTTO). Serum, gut and lung wash samples were diluted in BLOTTO, and 50µl volumes were assayed in duplicate in a series of twofold dilutions. After incubation overnight at 4°C, plates were washed three times in PBS with 0.02% (v/v) TWEEN 20™ (Polysorbate 20) ~~tween-20~~. Peroxidase-conjugated secondary antibodies against mouse IgG or IgA (Harlan Sera-Lab Ltd, Loughborough, UK), diluted 1:2000 in BLOTTO were incubated for 1 ~~hour~~ h at 37°C. The plate was washed as previously and 100µl of 2,2'-azino bis(3-ethybenzthiazoline-6-sulfonic acid) substrate (ABTS; Sigma, Poole, UK) was added. Antibody titre was estimated as the maximum dilution of serum giving an absorbance _{414nm} reading 0.1 U above background (Sera from animals immunized with SL3261 alone).